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Field evaluation of new olive (*Olea europaea* L.) selections and effects of genotype and environment on productivity and fruit characteristics

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Key words: breeding, environmental effects, heritability, olive and oil production.

Abstract: Rapid technological changes in olive growing have increased interest in breeding programs and new cultivars. A breeding program aimed at selecting new dual purpose (i.e. oil and table olive) cultivars began in Italy in 1971. In this paper we describe the agronomic performance (i.e. fruit and oil yield, fruit size, pulp to pit ratio, and oil content) of 134 olive selections grown in three locations of central and southern Italy. Twenty-one genotypes were selected as candidates for possible new cultivars based on their higher yield and yield efficiency. Data on many genotypes allowed assessment of variability of the studied traits in olive. The presence of many genotypes at all three locations, having different climates, allowed a quantitative analysis of the environmental (E^2) vs. the genotypic (heritability: H^2) effects on some traits in olive. Both environment and genotype had significant effects on all parameters tested. E^2 was greater than H^2 for average fruit weight and oil content on a fresh matter basis, while oil content on a dry matter basis and pulp to pit ratio were mainly under genetic control.

1. Introduction

Olive cultivation is moving from traditional, marginal areas to new areas where modern technologies can be used for mechanical pruning and especially harvesting. This change is occurring both in traditional olive-producing countries and in new countries where olive growing is rapidly expanding. This large scale phenomenon calls for new olive cultivars which are better adapted to the new technologies. Geneticists are working on new cultivars with early, uniform and high yield, suitability to mechanical harvesting and improved oil quality in addition to resistance to biotic and abiotic stress and ability to root from cuttings.

Breeding programs are currently being carried out in Tunisia (Jardak, 2006), Egypt (Laz, 2006), Israel (Lavee, 2006), Syria (Al Ibrahem, 2006), Turkey (Zafer Can and Isfendiyaroglu, 2006), Spain (Rallo, 2006) and Iran (Zeinanloo, 2006) where new genotypes

from cross-breeding programs are being evaluated. Results from such programs are still modest compared to those normally obtained with other species where many new cultivars are released in a short time. This is due to high heterozygosity and lack of knowledge about trait heritability in olive (Bellini *et al.*, 2003 a), long juvenility of the species and the many traits to be selected all at once. The only traits for which heritability has been studied in olive are plant height and shoot diameter (Martins *et al.*, 1998), and limited to clonal selection of Cobrançosa. No information exists for other traits of agronomic interest.

A cross-breeding programme was initiated at the University of Florence in 1971 (Bellini, 1993) by crossing 12 table olive and five oil cultivars, for a total of 127 crossings. Table olives were used in the programme in the hope of selecting new dual purpose, as well as either oil or table olive varieties. From the resulting 5000 seedlings, 134 were selected, propagated and planted in three locations of central and southern Italy (Bellini *et al.*, 2002 a, b, 2003 b). The initial selection work led to the release of three new cultivars, 'Arno',

'Tevere' and 'Basento', all derived from the Picholine x Manzanilla crossing (Bellini *et al.*, 2004). More recently, an initial agronomic evaluation of the selected 134 genotypes grown in three locations has been carried out (Padula *et al.*, 2006 a; Pannelli *et al.*, 2006) together with an initial evaluation of the oil quality in two of the three locations (Padula *et al.*, 2006 b).

In the present paper, further and more comprehensive data on the agronomic performance of the 134 olive selections grown in three locations of central and southern Italy are analyzed with the aim of selecting the best genotypes for the different possible uses (i.e. oil and table olives). Additionally, a quantitative assessment of the environmental (E^2) vs. genotypic (heritability: H^2) effects on the studied traits was carried out.

2. Materials and Methods

The 134 olive selections were propagated by self-rooted cuttings and planted in 1989 in three locations of central and southern Italy: Spoleto (42° 48' 48''N, 12° 39' 15''E, 356 m above sea level), Rossano Calabria (39° 36' 22'' N, 16° 38' 28'' E, 27 m above sea level) and Metaponto (40° 23'25'' N, 16° 46' 47'' E, 8 m above sea level). The soil was mainly clay in Spoleto, sandy in Rossano and of intermediate characteristics in Metaponto. In the latter location, trees were irrigated during the dry period (June-September) while in Spoleto and Rossano there was no irrigation. Plants were spaced 5 x 5 m (4 x 5 in Rossano) and trained to a vase. Of all genotypes, 38 of them were common to all three locations. Olive yield was measured in Spoleto in 1995-2006, except for 2000 and 2003; fruit characteristics were evaluated in the period 1996-1998 and in 2006. In Metaponto, both yield and fruit characteristics were measured in the period 1994-1997, and in 2000 and 2006. In Rossano these data were collected in 2005-2006. Tree growth in all locations was evaluated at the end of 2006, by measuring trunk cross-sectional area at 30 cm from the soil.

The following parameters were evaluated in mid-November:

- fruit yield, expressed as average annual yield per tree;
- oil yield, calculated from fruit yield and oil content;
- yield efficiency, both in olives and in oil, calculated as average yield/canopy volume at the end of 2006 (canopy volume was calculated as the volume of a cylinder with diameter = average of the two diameters measured between and within rows, and height = canopy height between the lowest and the highest parts of the vegetation);
- average fruit weight and pulp to pit ratio, calculated from fruit samples of at least 50 fruits;
- oil content both on a fresh and dry weight basis, initially assessed with the Foss-Let method, later with an Infrazyzer 2000 Olive (Bran+Luebbe, Germany).

Genotype suitability for the different uses (oil or table olives) was determined based on fruit oil content on a dry matter basis: above 48% oil, genotypes were considered suitable for oil; below 40% oil, for table olives; between 40 and 48% oil, for dual purpose (Servili *et al.*, 2006). For table olives and dual purpose cultivars, only genotypes with fruit weight > 2.43 g (IOOC, 2004) and pulp to pit ratio >5 were considered suitable.

The best genotypes were identified as those with better performance in terms of either yield or yield efficiency in either oil or fruits. Genotypes were chosen as those with average values greater than field average by more than two standard deviations.

The effects of the environment and genotype were analysed using available data from the genotypes common to all locations (n= 34). A two-way ANOVA was applied with genotype and location as the two variables, and using the data from different years as replications, except for trunk cross-sectional area which was measured in 2006 only and for which up to three different trees (when available) were taken as replications. The ANOVA was carried out using the R Development Core Team (2006) software.

3. Results and Discussion

Best genotypes

From all data pertinent to the 134 genotypes, 21 were selected for their superiority in one or more characters of productivity (Table 1 and 2). Genotype G XX 29 was selected in all fields for its high oil yield and it appears to be a promising genotype as a new cultivar with stable and high yields across different environments (Table 2). Genotypes G IV 22, P III 73 24 80 and P VI 73 were selected in two fields for at least one productivity parameter. The lack of common best genotypes (with the exception of G XX 29) suggests that it may be difficult to obtain new genotypes with high performances in different environments, and that best genotype-environment combinations, rather than best genotypes, may have to be pursued. Therefore, the other genotypes in Table 2 (i.e. the ones selected in only one field) may be of local interest, having performed well in given locations. This is especially true for those genotypes that were not present in all three locations, but were selected in all the locations where they were present: these genotypes should be tested in the other locations in the future.

Variability of traits among genotypes and locations

All measured traits had great variability among genotypes (i.e. several-fold differences between lowest and highest values), but also between locations (Fig. 1). Trees in Spoleto had lower yield (Fig. 1 A and B), while trees in Rossano were the most productive. Yield efficiency was lowest in Metaponto, the irrigated field, and similar between Spoleto and Rossano (Fig. 1 C and

Table 1 - Values for all the parameters measured for the best genotypes, in the locations where they performed best (see Table 2)

Genotype	Location	♀ x ♂	Main Use	Yield Fruits (g)	Yield Oil (g)	Yield Ef Fruits (g m ⁻³)	Yield Ef Oil (g m ⁻³)	Fruit Weight (g)	Pulp Pit (n)	Oil DM (%)	Oil FM (%)
G III 134	Rossano	Picholine x Coratina	Both	19850±600	3630±109	471.8± 93.8	86.3±17.2	2.5± 0.4	4.7±0.9	43.7± 1.9	18.3± 2.1
G IV 22	Spoleto	Picholine x Coratina	Both	5026±557	926±99	327.8± 127.1	60.4±4.9	3.1± 0.7	14.0±3.1	46.1± 4.1	18.4± 1.0
G IV 22	Rossano	Picholine x Coratina	Both	13225±265	2675±53	172.4± 3.1	34.9±0.6	2.6± 1.3	5.4±3.0	47.1± 3.8	20.2± 0.8
G X 88	Rossano	Picholine x Leccino	Both	18383±891	3929±190	655.8± 113.0	140.2±24.2	2.2± 0.4	5.9±2.0	44.6± 1.9	21.4± 2.2
G X 110	Spoleto	Picholine x Nocellara	Both	2347±24	488±5	469.9± 87.5	97.9±9.4	2.7± 0.5	5.3±0.4	43.8± 2.3	20.8± 2.3
G XII 36	Rossano	Grossane x Razzo	Oil	15916±1678	3566±376	210.7± 13.8	47.2±3.1	2.8± 0.1	5.5±1.1	53.1± 2.3	22.4± 2.6
G XIII 2	Spoleto	Grossane x Razzo	Both	1743±455	416±112	259.5± 27.5	62.0±3.7	3.0± 0.2	5.2±0.6	42.4± 2.7	23.9± 2.0
G XVIII 39	Spoleto	Leccino x free poll.	Both	2460±326	569±124	263.4± 42.2	60.9±2.5	3.0± 0.4	5.0±0.2	47.7± 0.7	23.1± 0.4
G XX 29	Spoleto	Leccino x Gordales	Both	5405±662	1115±136	224.3± 30.6	46.3±0.7	2.6± 0.2	4.3±0.3	46.1± 2.8	20.6± 1.1
G XX 29	Metaponto	Leccino x Gordales	Both	12141±798	1491±98	310.0± 19.6	38.1±2.4	4.3± 0.4	6.6±0.3	40.4± 1.1	12.3± 0.9
G XX 29	Rossano	Leccino x Gordales	Both	21075±2878	3884±530	334.2± 15.3	61.6±2.8	2.7± 0.5	4.4±1.4	44.8± 4.2	18.4± 2.1
G XX 31	Metaponto	Leccino x Gordales	Both	14134±277	1895±37	421.4± 53.4	56.5±7.2	4.1± 0.5	6.5±0.4	41.3± 1.6	13.4± 1.2
P I 3	Metaponto	Boutellain x Frantoio	Both	16352±714	2015±88	551.0± 38.7	67.9±4.8	4.1± 0.5	7.2±0.3	42.8± 1.5	12.3± 2.0
P III 73 24 80	Spoleto	Boutellain x Gordales	Table	3521±35	622±6	133.3± 28.2	23.6±1.4	3.7± 0.3	4.2±0.3	36.7± 4.3	17.7± 2.4
P III 73 24 80	Metaponto	Boutellain x Gordales	Table	5938±2711	749±342	291.8± 26.4	36.8±3.6	5.3± 0.4	5.7±0.2	37.8± 0.4	12.6± 0.2
P III 79	Metaponto	Boutellain x Coratina	Table	12427±728	1390± 81	389.6± 52.9	43.6±5.9	4.1± 0.9	8.1±0.3	35.3± 2.3	11.2± 0.1
P IV 6	Metaponto	Boutellain x Coratina	Both	8095±700	1426±123	515.3± 120.0	90.8±21.1	5.2± 0.5	6.4±0.2	44.8± 0.8	17.6± 1.3
P IV 72	Spoleto	Boutellain x Picholine	Both	5687±1235	1097±258	248.3± 36.8	47.9±6.5	3.6± 0.4	5.6±0.1	44.3± 2.1	19.3± 0.2
P V 11	Spoleto	Boutellain x Picholine	Both	4978±986	883±214	288.8± 25.8	51.2±4.6	3.4± 0.3	7.3±1.0	43.3± 2.7	17.7± 1.5
P V 18	Metaponto	Boutellain x Picholine	Oil	8948±614	1732±118	292.1± 6.8	56.6±1.3	5.6± 0.9	9.9±0.8	51.4± 0.1	19.4± 0.3
P V 93	Metaponto	Boutellain x Sorba	Both	12401±795	2146±137	583.7± 88.0	101.0±15.2	5.1± 1.0	7.6±0.8	47.1± 0.8	17.3± 1.2
P VI 73	Spoleto	Boutellain x Grossane	Both	3053±450	687±101	403.4± 65.8	90.8±1.3	3.6± 0.1	5.3±0.5	45.3± 0.9	22.5± 1.0
P VI 73	Metaponto	Boutellain x Grossane	Oil	15955±892	3051±170	529.6± 13.7	101.3±2.6	4.9± 0.2	6.3±0.1	48.7± 1.0	19.1± 0.3
P VIII 61	Rossano	Verdale x Leccino	Oil	19800±1493	3511±264	491.0± 62.4	87.1±11.1	2.8± 0.1	4.8±0.6	48.4± 2.9	17.7± 3.4
P XIV 92	Spoleto	Tanche x Botellain	Table	1708±236	295±40	407.5± 55.4	70.6±4.0	4.7± 0.2	6.5±0.5	38.7± 2.5	17.3± 2.0
P XV 76	Spoleto	Gordales x Leccino	Oil	2438±762	607±128	279.8± 35.8	69.7±5.4	2.0± 0.1	3.6±0.1	48.3± 0.9	24.9± 1.3

±= Standard error.

Table 2 - Genotypes with superior fruit yield (Y_F) and/or yield efficiency (E_F), oil yield (Y_O), oil yield efficiency (E_O); np = genotypes not present in a field

Genotype	Spoleto				Metaponto				Rossano			
	Y_F	E_F	Y_O	E_O	Y_F	E_F	Y_O	E_O	Y_F	E_F	Y_O	E_O
G III 134	np				np				x		x	
G IV 22	x		x	x					x		x	
G X 88					np				x	x	x	x
G X 110		x		x								
G XII 36									x		x	
G XIII 2				x	np							
G XVIII 39				x	np				np			
G XX 29	x		x				x				x	
G XX 31					x	x	x					
P I 3					x	x	x	x				
P III 73 24 80	x					x			np			
P III 79					x	x						
P IV 6						x		x	np			
P IV 72	x		x		np				np			
P V 11	x		x						np			
P V 18							x	x				
P V 93					x	x	x	x				
P VI 73		x		x	x	x	x	x	np			
P VIII 61									x	x	x	x
P XIV 92	x											
P XV 76			x	np					np			

D) despite very different climates. Decreased yield efficiency in Metaponto might have been due to irrigation since water availability is known to increase growth more than yield (Lavee *et al.*, 2007).

The highest values of average fruit weight (Fig. 1 E) and pulp to pit ratio (Fig. 1 F) were observed in Metaponto and the lowest in Rossano. Oil content was highest in Rossano on a dry-matter basis (Fig. 1 G) and lowest in Metaponto on a fresh-matter basis (Fig. 1 H).

Effect of environment vs. genotype

To evaluate the effect of environment and genotype on the measured parameters, only the data for the genotypes common to all locations (n= 34) were considered. The variability of the values for the different parameters was nearly identical to that obtained for all genotypes and is not shown.

The ANOVA gave significant effects of both genotype and environment on all parameters considered (Table 3). However, there was significant interaction between environment and location for fruit yield. A significant interaction implies that the effect of the location was different for the different genotypes and must be evaluated separately for each one. In other words, the genotypes with, for instance, the highest yield were not the same in the different locations. Consequently, we selected the most productive genotypes separately in each field (Table 2).

Fruit weight, pulp to pit ratio and oil content on a dry and fresh matter basis had non-significant interactions, implying that the environmental effects were similar on all genotypes. For these parameters, the environmental effects were further studied by plotting field average values against either the cumulated degree-days from January 1 to harvest, or against the cumulated precipitations (+irrigation for Metaponto) from May to harvest, of each location (averages for the years of observation). The degree-days were calculated as $[(T_{\min} + T_{\max})/2] - 7.5$, where T_{\min} = minimum daily temperature in °C; T_{\max} = maximum daily temperature in °C; and 7.5 is the minimum temperature below which olive growth and metabolism can be considered negligible (Bongi, 2004).

Average fruit weight appeared to be positively correlated ($R^2 = 0.95$) with water availability but not with temperature (Fig. 2). Pulp to pit ratio was strongly ($R^2 = 0.99$) and positively related to water but weakly to temperature. Oil content on a dry matter basis was strongly ($R^2 = 0.90$) and positively related to temperature and less strongly ($R^2 = 0.59$) and negatively to water, suggesting that this parameter is less dependent on water availability, in agreement with previous suggestions (Farinelli *et al.*, 2003). Oil content on a fresh matter basis was strongly ($R^2 = 0.72$) and inversely related to water and not related to temperature.

Since both environment and genotype had significant effects with non-significant interactions on fruit

Table 3 - Analysis of variance of the effects of genotype and environment on fruit characteristics and yield

Source	df	Average fruit weight (g)		Pulp to pit ratio (n)		Oil content (% dry matter)		Oil content (% fresh matter)		Yield (fruits) (g)	
		M.S.	F value	M.S.	F value	M.S.	F value	M.S.	F value	M.S.	F value
Genotype	33	4.3	4.9***	17.3	6.1***	97	2.3**	34	1.9*	61395091	21***
Location	2	79.3	88.6***	110.6	39.2***	304	7.3**	559	31.0***	759905281	265***
Gen x Loc	66	1.1	1.3	2.7	1.0	51	1.2	16	0.9	30154373	11***
Error	103	0.9		-		-		-		-	
Error	54	-		2.8		-		-		-	
Error	76	-		-		42		-		-	
Error	76	-		-		-		18		-	
Error	191	-		-		-		-		2862611	

*, ** and *** P values at 0.05, 0.01 and 0.001 levels of significance, respectively.

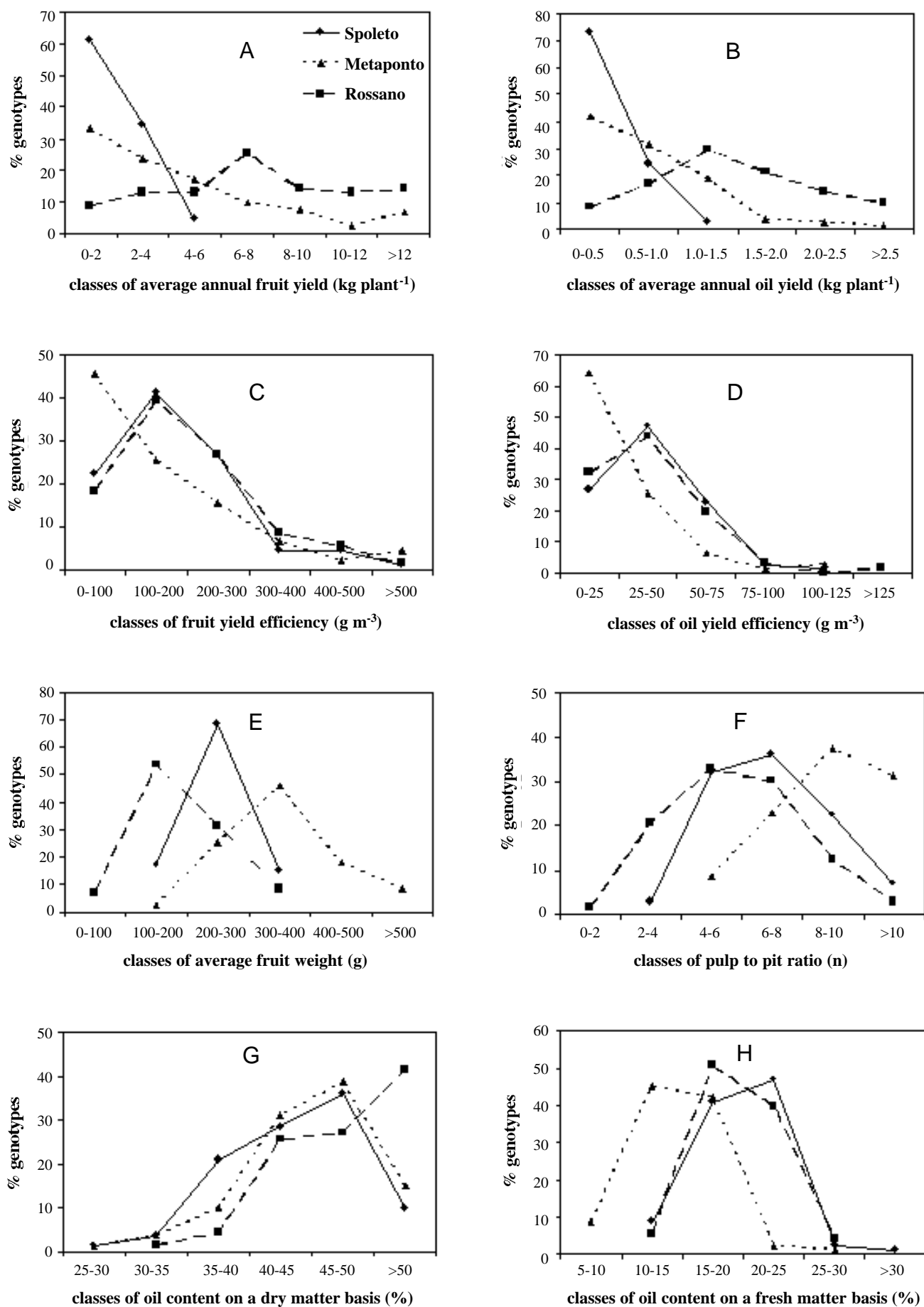


Fig. 1 - Genotype frequency distribution in classes of (A) average annual fruit yield per plant; (B) average annual oil yield per plant; (C) fruit yield efficiency; (D) oil yield efficiency; (E) average fruit weight; (F) pulp to pit ratio; (G) oil content on a dry matter basis; (H) oil content on a fresh matter basis.

weight, pulp to pit ratio and oil content (Table 3), the proportional importance of the two variables was calculated through a quantitative analysis. To this end, we calculated the fraction of the total variance of the data (for the genotypes common to all locations) or phenotypic variance (V_p) explained by the variance due to the location (V_L) or to the genotype (V_G). The varian-

ce explained by the genotype represents the broad sense heritability ($H^2 = V_G/V_p$). The variance explained by the environment is an estimate of the proportional weight of the environmental effects ($E^2 = V_L/V_p$).

These results show that the environment had quantitatively more impact than the genotype on fruit weight and oil content on a fresh matter basis, while pulp

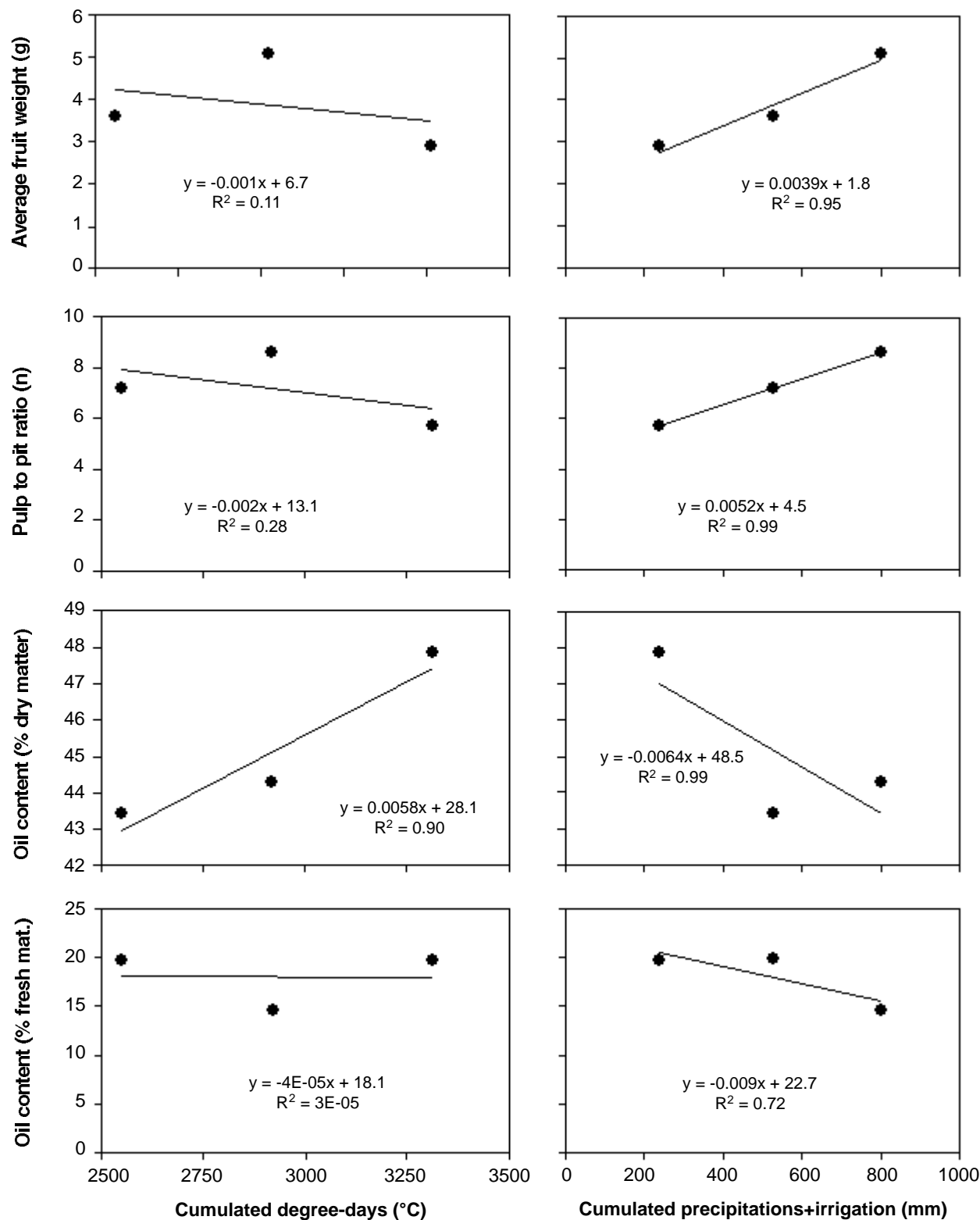


Fig. 2 - Relationship between fruit characteristics and the degree-days cumulated until harvest or the precipitations + irrigation cumulated from May to November.

to pit ratio and oil content on a dry matter basis were predominantly under genetic control and thus more heritable (Table 4). These findings agree with previous suggestions that oil content (on a dry matter basis) is less variable across environments and more genotype-dependent (Lavee and Wodner, 1991; Mickelbart and James, 2003; Lavee *et al.*, 2007). Pulp to pit ratio also appeared mostly under genetic control, despite being usually related to fruit weight, which was predominantly under environmental control. This is not contradictory since, despite being mainly under environmental control, fruit weight also had high H^2 (i.e. 0.40).

Table 4 - Fraction of the total variance explained by the genotype (H^2 : broad sense heritability) and environment (E^2)

	Average fruit weight (g)	Pulp to pit ratio (n)	Oil content (% dry matter)	Oil content (% fresh matter)
H^2	0.40	0.58	0.42	0.29
E^2	0.58	0.32	0.13	0.49

While the heritability of plant height and shoot diameter had been partially studied previously in a clonal selection of the olive cultivar Cobrançosa (Martins *et al.*, 1998), and while the heritability vs. environmental effects on olive acidic composition and phenolic content have been recently studied (Ripa *et al.*, 2008), the present data provides the first quantitative analysis of the heritability vs. the environmental effect on yield, fruit size and oil content in olive. This information will be useful for future breeding programs.

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